

Dielectric Relaxation Spectroscopy of Some Polysaccharides

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Dielectric relaxation spectra of four polysaccharides (hyaluronic acid extracted from rooster combs in the form of sodium salt, chondroitin sulfate C extracted from shark cartilage, hydroxyethylcellulose Natrosol H, and carboxymethylcellulose Blanose D) were studied by the time-domain dielectric relaxation spectroscopy in the frequency range 10^{-5} to 10^{-1} Hz. Observed spectra were interpreted as a sum of one conductivity process and one or two relaxation processes using the Havriliak—Negami formula. Numerical values of the parameters of conductivity and relaxation processes were calculated and the physical interpretation of the observed data was discussed.

Polysaccharide-based materials are widely used for many applications in medicine and biology. Physicochemical and physiological properties of polysaccharides are strongly determined by their primary structure and stereochemistry of the glycosidic bond as well as by the side groups, which are attached to the main chain. These groups are mostly of polar character and therefore their molecular dynamics can be followed by means of dielectric relaxation spectroscopy (DRS), either in the time domain (measurements of discharge currents), or in the frequency domain (measurements of complex permittivity).

Modified polysaccharides are carbohydrates, in which several different functional side chain groups and stereogenic centres are present in the main macromolecular chain (*e.g.* carboxyl, carbonyl, etheric, sulfate, acetylamidic, and hydroxyl groups) [1]. Important role of carbohydrates was confirmed in the field of recognition and selecting processes in organisms on biological membranes [2]. For example, selective interaction of carbohydrates with receptors is regulating the uptake of the blood serum proteins into the cell [3] and intercellular transport of enzymes [4]. They affect spreading processes of the infection and the variety of other immunological processes [5, 6]. From the above-mentioned aspects, particularly hyaluronic acid seems to be very interesting, because of its presence in different cell structures and environments.

Dielectric properties of the side chain groups, responsible for a specific selective recognition potential were studied with the aim to gain information about the stereochemical and chiral properties of polysaccharides and to influence them in the controllable manner. It is known, that the programmable changes of hydrophilic and hydrophobic microdomains in the

microstructure of some monosaccharides involved in recognition processes are responsible for their selective recognition potential [7]. Formation of these microdomains is closely linked with the processes influencing the stereoselectivity of these carbohydrates based on the ability of coordination with transition metals. Such coordination triggers the specific changes in spatial configuration of the polymer. The latter changes of the configuration are consequently reflected in changes of the material characteristics (*e.g.* UV VIS spectral characteristics, viscosity, elasticity, density, optical density, electrical conductivity, dielectric losses, electrical capacity) which can be followed by mechanical, optical, and electrical measurements. This allows to transform these selective material responses to the changes of the environment of the polysaccharide-based sensing system into the digital form. The study of interaction of hyaluronic acid with collagen and its effect on consecutive sensitivity to the other proteinic structures is of particular importance.

This paper presents the results of dielectric studies of unmodified polysaccharides in the low-frequency region at the ambient temperature.

EXPERIMENTAL

Four different types of polysaccharides were studied, namely hyaluronic acid extracted from rooster combs in the form of sodium salt ($M_r = 1.3 \times 10^6$ — 2.0×10^6) (Sigma), chondroitin sulfate C extracted from shark cartilage, approx. 90 % ($M_r = 58.8 \times 10^3$) (Sigma), hydroxyethylcellulose Natrosol 250 H (Hercules), and carboxymethylcellulose Blanose D in the form of sodium salt ($M_r = 0.55 \times 10^6$) (Hercules).

Dielectric spectra were obtained from the time-

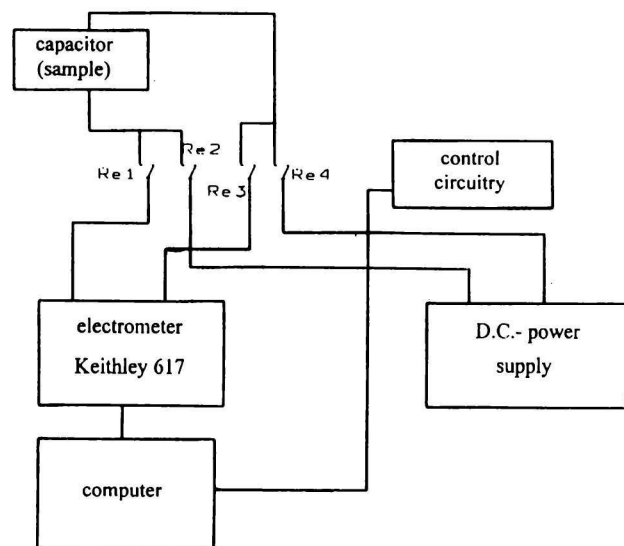


Fig. 1. Block scheme of the measuring apparatus.

domain measurements, *i.e.* from the measurements of the discharge current following an application of the D.C. electric field across the sample. The principal scheme of the measuring setup is shown in Fig. 1. The apparatus consists of a digital electrometer Keithley 617, stabilized D.C.-power supply, relay control circuitry, and personal computer interconnected *via* IEEE-488 bus. The measurements were fully computer-controlled and the only manual operation necessary was the setting of the charging voltage. The measurement consisted of two basic parts: charging and discharging periods. In order that the observed response may be corresponding to the properties of the material under study, the discharge period must be at least three times shorter than the corresponding charging period [8]. For the majority of the measurements, the charging period was set to be of 24 h, so that the corresponding discharge period might be of 8 h. Since at present the measurement of the discharge current starts at the time of 1 s after the beginning of discharging, the period starting from 1 s to about 10^4 s is covered by these measurements.

Prior to charging, samples were kept short-circuited for at least 12 h in order to remove any stray charges. Every data point was taken twice: first the discharge current was measured in the automatic range regime and the value of the discharge current was taken in the most suitable fixed range. Afterwards the measuring range was switched over appropriately. The values of time and discharge current were stored in the form of a data file for further analysis.

Measured samples were of the circular or rectangular shape with graphite electrodes of the size about 12 mm × 20 mm pasted onto their surface. Samples were fixed in the sample holder and placed into the thermostatic chamber. Due to the hygroscopic nature of the samples the measurements were sensitive to the

humidity of the surrounding atmosphere; therefore the measuring chamber was provided with a simple installation based on the oversaturated solution of K_2CO_3 . This allowed to keep the humidity within the thermostatic chamber at constant value of 43 % in the course of the measurement.

Data Processing

The results of the time-domain measurements were obtained in the form of the time dependence of the discharge current. The time-domain and frequency-domain results are related between each other by the Fourier Transformation (FT). The time-domain data were transformed into the frequency domain and yielded the frequency dependence of the complex permittivity according to the formula [9]

$$\hat{\varepsilon}(\omega) = \varepsilon_\infty + (\varepsilon_s - \varepsilon_\infty) \int_0^\infty \varphi(t) \exp(-j\omega t) dt \quad (1)$$

or after the separation into the real (ε') and imaginary part (ε'')

$$\varepsilon'(\omega) = \varepsilon_\infty + (\varepsilon_s - \varepsilon_\infty) \int_0^\infty \varphi(t) \cos(\omega t) dt \quad (2)$$

$$\varepsilon''(\omega) = (\varepsilon_s - \varepsilon_\infty) \int_0^\infty \varphi(t) \sin(\omega t) dt \quad (3)$$

where $\varphi(t)$ is the decay function of the dielectric related to the discharge current $i(t)$ by the formula

$$i(t) = -\varepsilon_0(\varepsilon_s - \varepsilon_\infty) \frac{UA}{d} \varphi(t) \quad (4)$$

In the above formulas the symbols have their usual meaning; $\hat{\varepsilon}(\omega)$ is the frequency-dependent complex permittivity, ε_∞ optical permittivity of the sample (corresponding to the electronic polarizations), ε_s static permittivity of the sample (corresponding to the total polarization including dipole and interfacial contributions), $\varphi(t)$ decay function, U applied voltage, A sample surface area, and d sample thickness.

As for the analysis of dielectric processes the imaginary part of the complex permittivity is sufficient, it is necessary only to obtain $\varepsilon''(\omega)$ from the time-domain measurements. In view of the slowly decreasing discharge current with only small variations of the slope of the $i(t)$ curve in the logarithmic coordinates, it is possible to use a simplified form of the Fourier Transformation, the so-called Hamon approximation [10]

$$\varepsilon''(f) = \frac{i \left(\frac{0.1}{f} \right)}{2\pi C_0 U f} \quad (5)$$

where $f = \omega/2\pi$, C_0 is the geometrical capacitance of the sample and i discharge current.

Dielectric spectra were analyzed in terms of conductivity and relaxation processes. The contribution of the conductivity component was modelled using the formula

$$\epsilon_c''(\omega) = A'\{\omega\}^{-n} \quad (6)$$

where the value of n equal to one corresponds to the purely D.C. conductivity mechanism. The contribution of the relaxation process to the complex permittivity was modelled with the commonly used Havriliak—Negami formula [11, 12]

$$\hat{\epsilon}_r(\omega) = \epsilon_\infty + \frac{\epsilon_s - \epsilon_\infty}{[1 + (j\omega\tau_0)^\alpha]^\beta} \quad (7)$$

where τ_0 indicates the position of the relaxation on the time axis, $(\epsilon_s - \epsilon_\infty)$ denotes the relaxation strength, α describes the slope of the low-frequency side of the relaxation, and the product $\alpha \times \beta$ describes the slope of the high-frequency side of the relaxation.

Dielectric spectra obtained by means of eqn (5) were fitted with a sum of up to two conductivities and two relaxation processes described by formulas (6) and (7). The approach chosen for fitting was based on the nonlinear least-squares method using the Levenberg—Marquardt algorithm (implemented in the PASCAL fitting program).

RESULTS AND DISCUSSION

Time dependences of the discharge current of all samples are shown in Fig. 2. These plots exhibit a steady decrease of the discharge current with time. The slope of the decrease is changing, which suggests a presence of a relaxation process. The charging voltage used for the measurement was 3 V, as the application of higher voltages brought about an appearance of the local maximum in the $i(t)$ curve (Fig. 3). As this local maximum vanishes when charging voltage is reduced, its origin was ascribed to the heating of the sample during the charging and subsequent cooling with accompanying moisture intake during the discharge period. Consequently, all measurements were carried out at voltages below 10 V, where this effect was absent.

Essential issue of the experiments was the reproducibility of the measurements. Fig. 4 shows the $i(t)$ curves taken repeatedly for the same sample of hyaluronic acid. Apparently, the measurements are not quite identical, but the differences are rather small (calculated standard errors of these measurements were of $\pm 3.5\%$). However, these differences increase considerably once the humidity in the measuring chamber starts to fluctuate. Observed minor differences in the measured data shown in Fig. 4 were ascribed to the fluctuations of the humidity conditions in the measuring chamber. As it is known, these conditions are strongly affecting the swelling behaviour of hyaluronic acid, thus affecting the mobility of counterions and skeletal macromolecular segments of the polymer matrix.

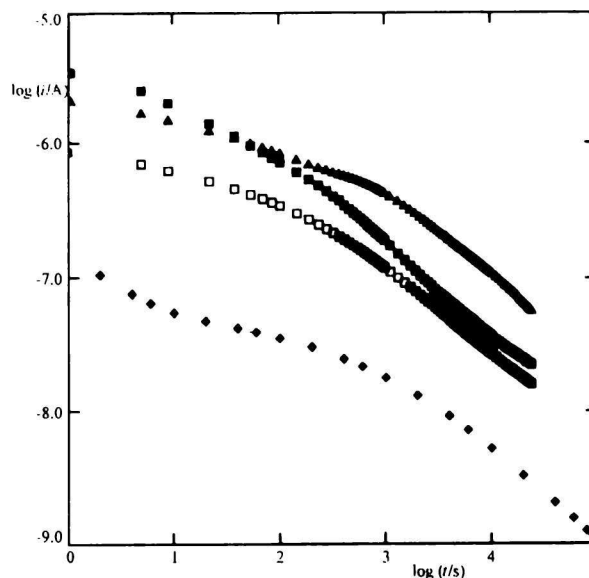


Fig. 2. Time dependence of the discharge current for hydroxyethylcellulose (open squares), chondroitin sulfate (full diamonds), carboxymethylcellulose (full triangles up), and hyaluronic acid (full squares), charging voltage 3 V.

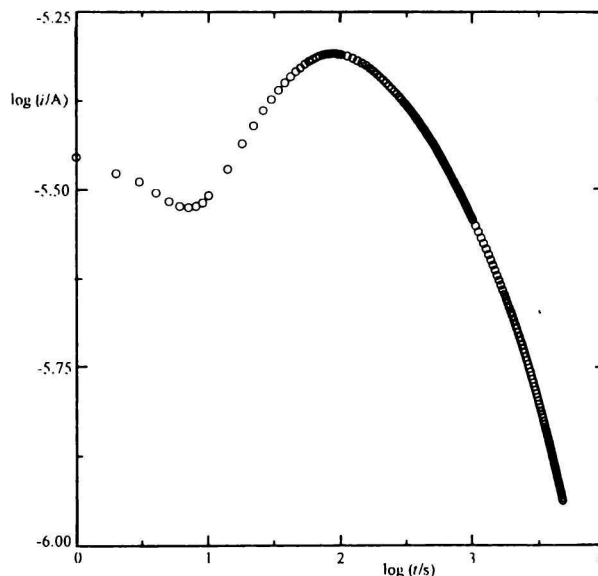


Fig. 3. Time dependence of the discharge current for hydroxyethylcellulose (open circles), charging voltage 66 V.

The relaxation spectra, *i.e.* the plots of the frequency dependence of the imaginary part of the complex permittivity of the samples together with individual conductivity and relaxation components obtained from the fits are shown in Figs. 5—8; the results of the fits are summarized in Table 1. In view of eqn (5) frequency interval of the dielectric spectrum after the Fourier Transformation was 10^{-5} — 10^{-1} Hz. The values of A and of the relaxation strength $(\epsilon_s - \epsilon_\infty)$

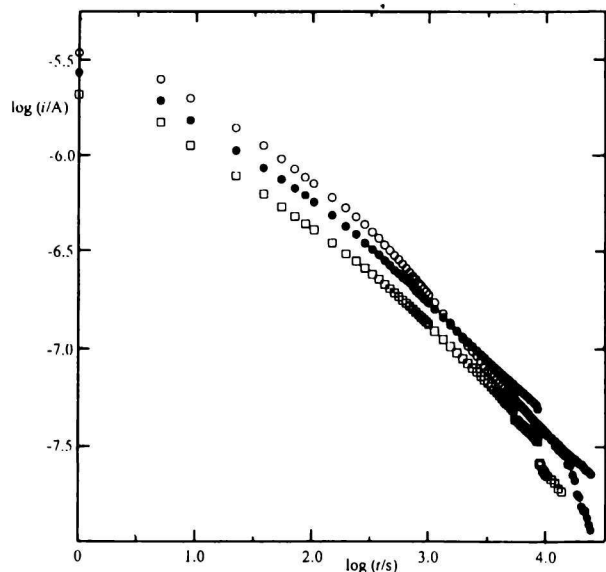


Fig. 4. Time dependence of the discharge current of hyaluronic acid after prolonged time period, first run – open circles, second run – full circles (9 days later), third run – open squares (10 days later).

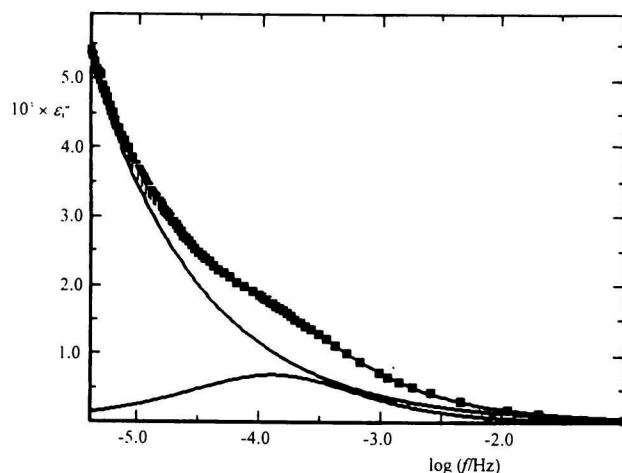


Fig. 5. Dielectric spectrum of the hyaluronic acid: measured data (full squares), total fitted spectrum and its components (solid line).

are only relative values, since they do not take into account different thickness of the measured samples, which appears in eqn (5) in the geometrical capacity term C_0 . As can be seen from the plots, in most cases one relaxation process and one conductivity component are sufficient for a reasonable agreement between the fit and the measured results. Only in the case of the carboxymethylcellulose the fit with one relaxation process was apparently not satisfactory enough to reach the optimum minimization conditions, therefore the fit with two relaxation mechanisms was necessary to proceed (Fig. 8, Table 1).

Value of the exponent n in eqn (6) was for all sam-

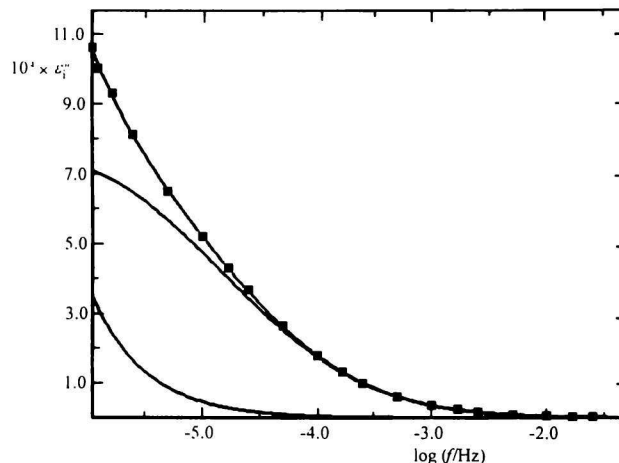


Fig. 6. Dielectric spectrum of the chondroitin sulfate: measured data (full squares), total fitted spectrum and its components (solid line).

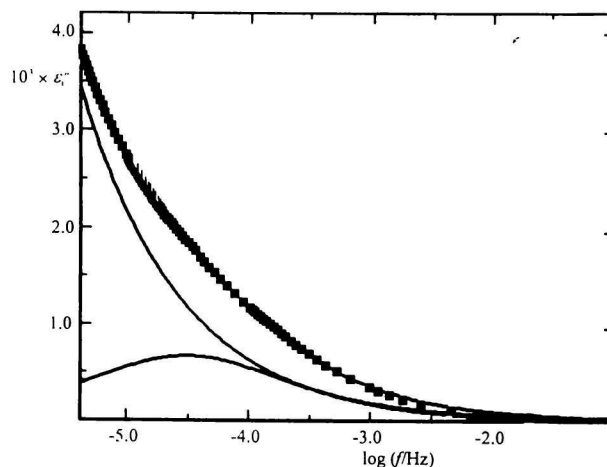


Fig. 7. Dielectric spectrum of the hydroxyethylcellulose: measured data (full squares), total fitted spectrum and its components (solid line).

ples studied lower than 1, which indicates the presence of mechanism of A.C. conductivity rather than D.C. conductivity [11]. The origin of the A.C. conductivity is usually interpreted as being due to the motion of loosely bound electric charges which form temporary neutral pairs during the conduction process. These neutral pairs have no electric charge and therefore during the period of their temporary existence they do not contribute to the conductivity, which is reflected in the decrease of the exponent n in eqn (6).

Scaling parameters are related to the molecular mobility and reflect the existence of some forms of ordering in the molecular system. Parameter α is known to decrease with the increase of the intermolecular interactions and long-range correlation of segmental motions [13]. Hence, the intermolecular interaction and long-range correlation are the strongest in chondroitin sulfate ($\alpha = 0.291$) and the weakest in hyaluronic acid

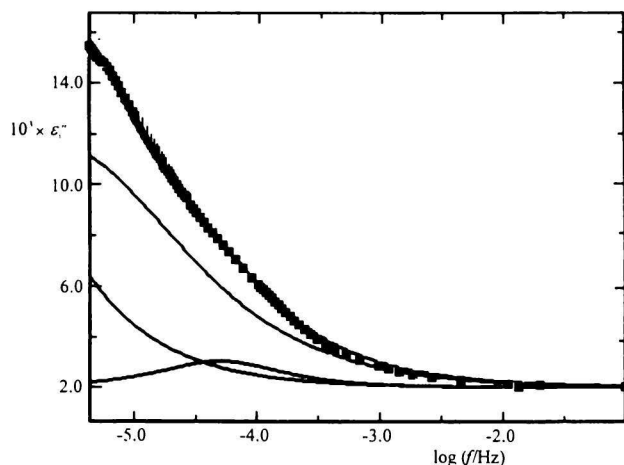


Fig. 8. Dielectric spectrum of the carboxymethylcellulose: measured data (full squares), total fitted spectrum and its components (solid line).

($\alpha = 0.726$). This observation is not surprising, because only the molecule of chondroitin sulfate (from all samples studied) is bearing two groups of a strong polarity in its monomeric unit, *i.e.* the carboxyl group and sulfate. These groups are responsible for the creation of an effective *intra-* and *inter-*molecular hydrogen bonding system in the molecule. Similarly, the product $\alpha \times \beta$ decreases with the increase of hindrances for conformational transitions in the polymer chain. Therefore, the effect of steric hindrances seems to be most pronounced in the case of hydroxyethylcellulose ($\alpha \times \beta = 0.676$). This is most probably due to the presence of the long side chain groups in the monomeric unit of studied hydroxyethylcellulose Natrosol 250 H. These side groups are build up of two ethylene oxide chains attached each other on one hydroxyl group in

the cellulose chain. The presence of such side chains is proposed in the idealized structure of the Natrosol 250 hydroxyethylcellulose by the manufacturer [14].

Observed relaxation times τ_0 for all samples are quite close to each other and are all of the order of 1000 s, although the side groups of individual polysaccharides are different. Therefore the origin of the measured relaxation is ascribed to the large scale rearrangements of the main macromolecular chain. It can be proposed that the relaxation times of such a large scale rearrangements would not be much different because of the high similarity of the macromolecular main chain structural components of the studied polymers. The relaxation processes induced by the motion of the different side groups should be different, but they will occur at much higher frequencies (above 1 Hz). The behaviour at higher frequencies is under study.

CONCLUSION

To achieve acceptable reproducibility (standard error of about $\pm 3\%$) of the measured results of the time-domain dielectric relaxation spectroscopy requires to keep constant humidity conditions in the measuring chamber. Differences between the dielectric relaxation spectra of the polysaccharides studied are relatively small in the frequency range of 10^{-5} –1 Hz. Dielectric relaxation spectra may be described as a sum of one A.C. conductivity mechanism and at least one relaxation process with the corresponding relaxation time τ_0 of the order of 10^3 s.

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Table 1. Calculated Parameters of Measured Dielectric Spectra of Studied Polysaccharides

Parameter	Compound			
	Hyaluronic acid	Chondroitin sulfate C	Hydroxyethylcellulose	Carboxymethylcellulose
$10^3 (\epsilon_s - \epsilon_\infty)$	2.05	4.77	2.28	37.60
α	0.726	0.291	0.676	2.18
β	1.155	4.010	1.000	0.598
$10^{-3} \tau_0/s$	1.020	3.440	5.120	0.970
A'	3.16×10^{-18}	5.58×10^{-22}	1.08×10^{-18}	1.000
n	0.473	0.919	0.535	68.100
				3.810
				2.48×10^{-19}
				0.700

ϵ_∞ optical permittivity of the sample, ϵ_s static permittivity of the sample, $(\epsilon_s - \epsilon_\infty)$ relaxation strength, τ_0 position of the relaxation on the time axis, α slope of the low-frequency side of the relaxation curve, product $\alpha \times \beta$ describes the slope of the high-frequency side of the relaxation curve, A' and n are the parameters from eqn (6).

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